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REVIEW

Different doses of phosphorus, calcium, and vitamin D in premature infants and their effect on bone mineralization: systematic review and meta-analysis

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Abstract

Purpose This systematic review and meta-analysis aims to assess the impact of different doses of Ca, P, and vitamin D on bone mineralization in premature infants.

Methods A systematic search (1990–2022) of MEDLINE (Ovid), Cochrane Library, Scopus, EMBASE, and CINAHL (EBSCO) was conducted. Randomized control trials and cohort studies, involving premature infants with birthweight ≤2.5 kg, who received supplementation and underwent bone mineral content reassessment, were included. Impact on growth indicators was also evaluated, but not systematically. Following the critical evaluation process, using PEDro scale and JBI critical appraisal checklist, high-quality studies were reviewed. Random effect meta-analyses (standardized mean difference) were performed to assess the effect of increased doses of Ca, P, and Vitamin D on bone health indicators.

Results Eighteen studies were included, fifteen with enteral and three with parenteral nutrition regimen. The included studies' mineral intake ranges presented high heterogeneity. The beneficial effect of higher doses of Ca and P on bone mineralization was evident in most studies, and unanimous when accompanied with higher doses of vitamin D, indicating the synergist effect of the three elements. Higher enteral nutrition doses of (a) Ca and P or (b) Ca, P, and vitamin D resulted in increased bone mineralization (standardized mean difference: 0.39; 95% CI 0.09, 0.69, and 1.72; 0.81, 2.16), respectively, while higher supplementation of only vitamin D presented no such effect (−0.01; −0.59, 0.56). Higher parenteral nutrition doses of Ca and P proved beneficial for bone mineralization (0.88; 0.34, 1.43). Higher enteral doses of all elements indicated no additional effect on growth.

Conclusions Elevated intake of Ca (daily doses: Ca 95–135 mg/100 kcal) and P (55–95 mg/100 kcal) throughout enteral nutrition together with sufficient vitamin D intake might prove beneficial towards enhancing bone mineralization in preterm infants.

Keywords Calcium · Phosphorus · Vitamin D · Bone mineralization · Bone health · Premature infants

Abbreviations

AAP	American Academy of Pediatrics
BTT	Bone transmission time
Ca	Calcium
DXA	Dual-energy X-ray absorptiometry
ELBW	Extremely low birth weight
EN	Enteral nutrition
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
HG	High-dose group
LBW	Low birth weight
LG	Low-dose group
MBD	Metabolic bone disease
P	Phosphorus
PN	Parenteral nutrition
RCTs	Randomized control trials
SOS	Speed of sound
VLBW	Very low birth weight
WHO	World Health Organization

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Introduction

Prematurity can increase the risk of poor bone health and developing metabolic bone disease (MBD), by missing the last months of rapid mineral accretion [1]. World Health Organization (WHO) defines prematurity as gestational age less than completed 37 weeks [2], and classifies birthweight as low birthweight (LBW) <2500 g, very-low birthweight (VLBW) <1500 g, and extremely-low birthweight (ELBW) <1000 g [2]. MBD, a bone strength disorder, is characterized by abnormalities of phosphorus (P), calcium (Ca), and vitamin D homeostasis and reduced bone mass and structure [1], and is expected to present in 16–40% of VLBW and ELBW infants [3, 4]. Even if MBD development is avoided, the effect of low birth weight and potentially poor bone health at birth due to prematurity can impact the quality of life and bone health for several years after birth [5, 6].

Suboptimal Ca and P intake can contribute to insufficient bone mineral density (BMD), a precursor to prematurity-attributed MBD [7], while insufficient Vitamin D intake can further diminish bone density and bone mineral content (BMC) of Ca and P. Therefore, adequate intake of these elements is suggested for primary prevention of MBD, administered through intravenous route, feeding tube or orally (breastmilk, fortified human milk, and formula) [8]. Magnesium also plays a fundamental role in the bone matrix structure, but during prematurity, its role is substantially less important [9].

The recommended supplementation of Ca, P, and vitamin D in premature infants vary among health organizations. In particular, the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) suggests an enteral nutrition (EN) intake of Ca: 120–200 mg/kg/day and P: 70–115 mg/kg/day [10]. In contrast, the American Academy of Pediatrics (AAP) recommends an intake of Ca: 180–220 mg/kg/day and P: 100–130 mg/kg/day, mainly through fortified breast milk and preterm formula [11]. Vitamin D recommendations also vary; ESPGHAN has recently changed its recommendation from 800–1600 IU/day to 400–700 IU/kg/day with a maximum dose of 1000 IU/day, while AAP recommends 400 IU/day for LBW infants and 200–400IU/day for VLBW infants [10, 11]. As for the Parenteral Nutrition (PN), the range of these nutrients recommended doses (ESPGHAN) are smaller for calcium and higher for vitamin D [12, 13].

A recent systematic review [14] examined the various formulas' effect on growth and bone mineralization in prematurity but not the exact doses of each mineral. To cover the literature gap, we aimed to systematically define the most beneficial doses of Ca, P, and vitamin D in premature infants with non-optional birthweight, comparing different doses (EN and PN). Secondary objectives were to assess the effectiveness of those elements on weight, length, and head circumference.

Methods

Registration

The review was conducted in accordance with the PRISMA statement [15]. The review protocol was registered with the International prospective register of systematic reviews (PROSPERO); registration number: CRD42022321481 (4/14/2022).

Inclusion criteria

Participants: Infants born prematurely (<37 weeks), with at least LBW (<2.5 kg) [16]. Despite MBD being more likely to develop in VLBW infants, we included infants of LBW as well, to assess the outcome of bone health in high-risk premature infants. Studies with a sample of only MBD infants or other diseases affecting the elemental/vitamin metabolism, like kidney disease, were excluded.

Intervention: Studies that use supplementary/higher doses of Ca, P, and/or vitamin D administered through EN (preterm infant formulas or expressed breast milk) or PN.

Comparator: Control group with no supplementation or lower doses of Ca, P, and/ or Vitamin D.

Main outcomes: Bone mineralization evaluated by dual-energy X-ray absorptiometry (DXA), speed of sound (SOS), or bone transmission time (BTT), an inexpensive accurate measure of organic matrix and BMC [8]. Serum biochemical biomarkers (ALP, Ca, P) were excluded for not being specific MBD predictors and accurate measures of BMC in prematurity [8]. Measurements taking place until 24 months of life were included because bone mineralization could still be increased during this time frame [17].

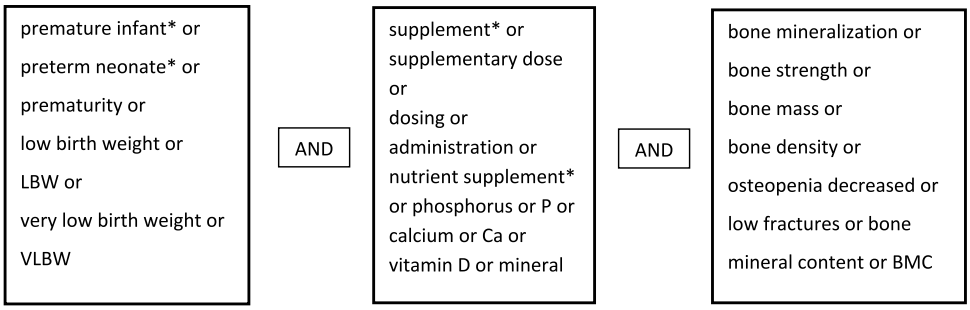
Secondary outcomes: Weight gain, length accrual (measured by electronic baby scale and infant measuring, respectively), and head circumference (by flexible non-stretch tape). These outcomes were not inserted into the search strategy and were not systematically assessed.

Type of studies: Randomized control trials (RCTs) and prospective or retrospective cohort studies. Conference abstracts and expert opinions were excluded.

Search strategy

Scientific databases searched included MEDLINE (Ovid), Cochrane Library, Scopus, EMBASE, and CINAHL (EBSCO). The search terms used are presented in Fig. 1.

Fig. 1 Literature search terms



Publication dates were limited between January 1990 and April 2022, without language restriction. Other sources searched include ClinicalTrials.gov, theses, conference abstracts, OpenGrey, Google Scholar, and WorldWideScience.org. A full search strategy example for Ovid MEDLINE database is shown in Supplementary Table 1.

As the institutional full-access to these databases was restricted following April 2022, an update bibliographic search in COCHRANE Library and PUBMED, an interface used to search MEDLINE(Ovid), was performed (1/April/2023–1/March/2023), amounting to 314 manuscripts, of which none satisfied study selection criteria. These searches are not included in the PRISMA flowchart, but are included In Supplementary Table 2.

Study selection

Two independent reviewers conducted the study selection to minimize the study inclusion bias. Articles retrieved were uploaded to Rayyan software for screening, and duplicates removal [18]. Following a pilot screening test, if an article was judged as relevant by the title and abstract, full-text was retrieved, unless it was retracted, unavailable, or a conference abstract. Reference lists of included studies were hand-searched. A PRISMA flow diagram provides visual mapping of the search strategy (Fig. 2) [15, 19].

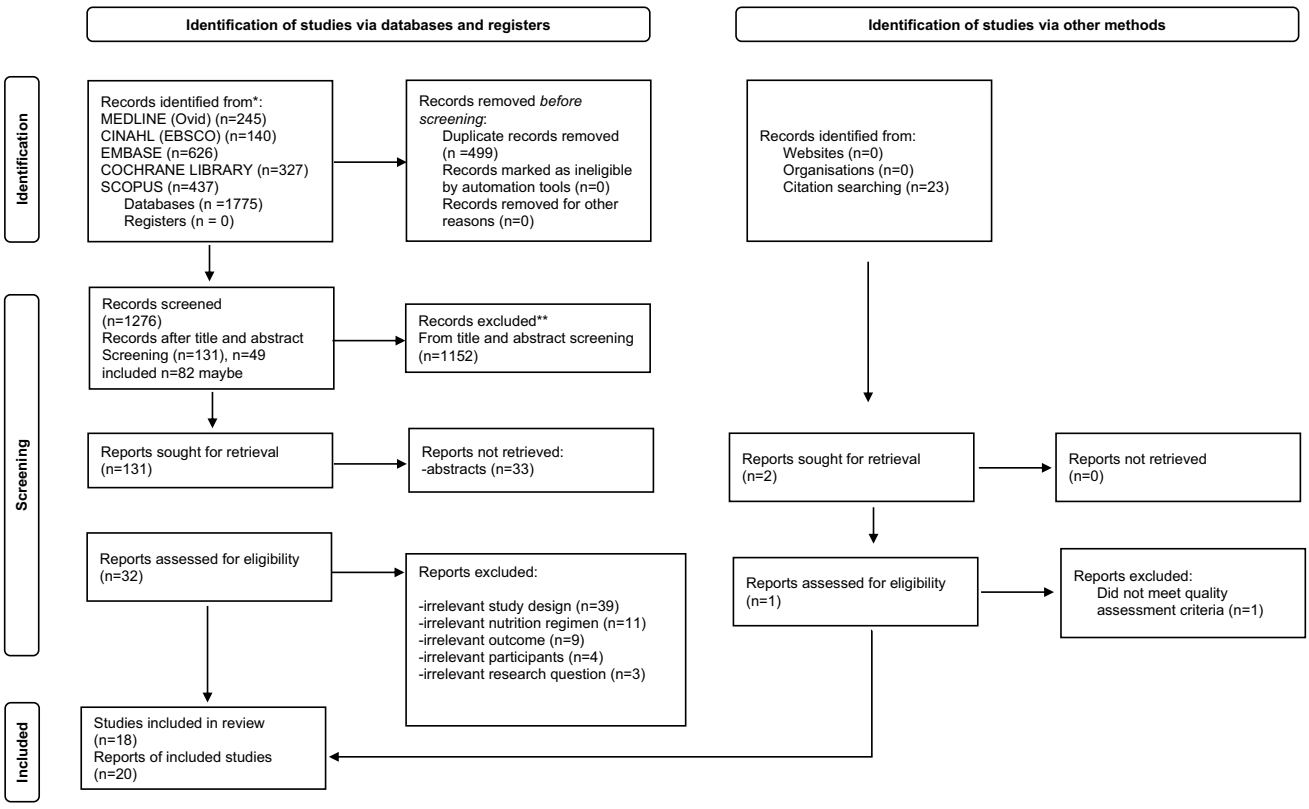


Fig. 2 PRISMA flow diagram

Assessment of methodological quality

Methodological quality was assessed through PEDro Scale for RCTs and JBI critical appraisal checklist for cohort studies by two reviewers [20–22]. PEDro scores were categorized as "poor" (0-3) "fair" (4-5), "good" (6-8), and "excellent" (9-10), a classification advised but not yet validated [21]. If an RCT scored ≥7, or a cohort scored ≥8 (answer "yes"), it was included and imported into data extraction [22].

Data extraction

Both reviewers used a standardized JBI data extraction form. To ensure consistency and validity, the following details were extracted: research details, participants' characteristics, intervention details (doses, type, and duration of administration and follow-up), outcomes assessed (bone health indicators, weight, length, and head circumference), and key findings.

Ca, P, and vitamin D content was translated to an average mg or IU per 100 kcal, whenever studies provided data on mean/exact volume intake. If not, the average mg or IU content per 100 kcal was calculated. Cross-calculations in human breast milk utilized the average content of kcal, Ca, P, and vitamin D content, as indicated by Kim and Yi [23]. Whenever median and IQR were presented, if there was some evidence of normal distribution, median was translated to mean (approximately equal) and IQR to SD, following the equation SD = IQR/1.35 [24].

Data synthesis and meta-analysis

Data synthesis followed a narrative presentation. Random effect meta-analysis was performed, categorizing the included studies into three categories: (A) higher Ca and P doses (not vitamin D); (B) higher Ca, P, and vitamin D doses; and (C) only higher vitamin D doses among groups. As there was inconsistency in the measured outcome (i.e., BMC measured in g and BMD in g/cm or g/cm^2, or g/cm^3) and in the method used to evaluate the same effect (i.e., BMC, BMD, SOS), a random effect standardized mean difference model was created for the included studies [24]. Additional models are presented in the supplementary material. The RevMan 5.4 software was used to perform the meta-analyses.

Results

Identified studies

The literature search yielded 1775 results (Fig. 2). After duplicate removal, screening, and hand search of citations and references, 20 full-text publications of 133 full-text screened were deemed eligible for inclusion, after methodological evaluation (Fig. 2). Faerk et al. [25] and Van de Lagemaat et al. [9] have published pertinent or duplicate results in later publications, which were excluded (the duplicates), resulting in 18 included studies (16 RCTs, one retrospective cohort, one prospective cohort study). Characteristics and key findings of included studies are presented in Table 1.

Assessment of bias

The methodological quality of the RCTs was considered either excellent ($n = 9$), or good ($n = 8$). Both cohort studies scored 8 "Yes," and were classified as excellent or good (Table 2).

Participant characteristics

The total number of participants was 991 (263 boys, 249 girls, and 479 unidentified sex). Mean gestational age ranged from less than 28 to 34 weeks. Birthweight of premature infants varied. The vast majority of the included sample included premature infants of less than 1500 g (VLBW), as most studies included only VLBW (9/18), mostly VLBW (7/18) or mostly LBW (2/18).

Doses of minerals and vitamin

Included studies, presented at least one comparator group, with higher administration dose of minerals (HGs), compared to the control groups or LGs. Ca and P administration (varying doses) was examined in 13 studies, with the HG dose in EN regimens including an average of 73–204 mg/100 kcal Ca and 49–105 mg/100 kcal P, and the LGs including 23–99 mg/100 kcal Ca and 20–63 mg/100 kcal P. In two PN regimens, the HG dose included an average range of 73–94 mg/100 kcal Ca and 43–87 mg/100 kcal P, and the LGs including 44–69 mg/100 kcal Ca and 25.8–62 mg/100 kcal P. In two studies either milk, bank or formula average volume was not reported [25] or only the supplemented parenteral doses were reported [26].

Vitamin D administration varied in 4/12 studies, with the daily enteral supplementation in the HG ranging from 800 to 1000 IU and in the LG from 200 to 500 IU. In 7/12 studies, the intervention group received enriched formula containing approximately 215 IU/100 mL, while the control group received a standard formula containing approximately 130 IU/100 mL. Koo et al. [27] compared the effectiveness of a nutrient-enriched formula containing 80IU/kcal with a term formula containing 60 IU/kcal.

Table 1: Descriptive analysis of included studies

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
1993	Bishop et al./UK	RCT	31	15 males 16 females	Premature infants with weight less than < 1850 g at birth and < 300 g gained at entry to the study, age < 100 days at discharge, no major congenital malformations.	Ca: 70 mg/100 mL (97 mg/100 kcal) (HG) versus 35 mg/100 mL (52 mg/100 kcal) (LG) P: 35 mg/100 mL (49 mg/100 kcal) (HG) versus 29 mg/100 mL (43 mg/100 kcal) (LG) Vitamin D: 48 IU/100 mL (HG) versus 40 IU/100 mL (LG)	HG: enteral preterm formula LG: standard enteral formula	9 months	BMC (mg/cm) Bone width (cm)	BMC was higher in HG compared to LG 3 and 9 months after discharge.	NA
1993	Chan, et al./USA	RCT	29	10 males 19 females	Premature infants	Ca: 120.8–136.4 mg/100 mL (180–204 mg/100 kcal) (HG1) versus 62.4–68.6 mg/100 mL (93–102 mg/100 kcal) (HG2) versus 53.5–55.9 mg/100 mL (80–83 mg/100 kcal) (LG) P: 61.6–70.3 mg/100 mL (92–105 mg/100 kcal) (HG1) versus 36.1–39.2 mg/100 mL (54-59 mg/100 kcal) (HG2) versus 42.5 mg/100 mL (63 mg/100 kcal) (LG) Vitamin D: 1250 IU/100 mL (HG1) versus 561–620 IU/100 mL (HG2) versus 400 IU/100 mL (LG)	HG1: enteral formula for premature infants HG2: enteral formula for low birth weight infants LG: standard enteral formula	16 weeks	BMC (mg/cm) Or BMD Weight gain Length gain	At 8 weeks BMC was higher in infants in HG1 than LG ($p < 0.001$). At 16 weeks, BMC had no difference among all groups.	Similar values in weight (HG: 5150 ± 204 g versus LG: 5020 ± 198 g) and length gain (HG: 1.23 ± 0.05 mm/day versus 1.19 ± 0.06 mm/day) remained similar among feeding groups after the 16 weeks from discharge ($p > 0.05$).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
1993	Prestridge et al./USA	Randomized control trial (RCT)	24	10 males 14 females	Premature infants with gestational age 27 ± 2 weeks, birth weight <1.2 kg, no major congenital malformations, and parenteral nutrition (PN) duration for approximately 3 weeks	Ca: 67 mg /100 mL (94 mg/100 kcal) (high group-HG) versus 50 mg/100 mL (69 mg/100 kcal) (low-dose group—LG) P: 62 mg/100 mL (87 mg/100 kcal) (high-dose group—HG) versus 45 mg/100 mL (62 mg/100 kcal) (low-dose group—LG)	PN	26 weeks	BMC (mg/cm)	Absolute BMC is significantly higher in HG compared to LG (74 ± 11 versus 88 ± 15 mg/cm). ($p = 0.03$) Total rate of increase in BMC significantly higher in HG compared to LG (2.0 ± 0.50 vs 2.7 + −0.6 mg/cm/week). ($p = 0.025$)	NA
1998	Alpay et al./Turkey	Cohort study	43	22 males 21 females	Preterm infants without intracranial hemorrhage, respiratory distress syndrome, major congenital anomalies, and total parenteral nutrition for more than 5 days.	Ca: 70 mg/100 mL (100 mg/100 kcal) (HG) versus 23 mg/100 mL (34 mg/100 kcal) (LG) P: 35 mg/100 mL (50 mg/100 kcal) (LG) versus 13 mg/100 mL (20 mg/100 kcal) Vitamin D: 85 IU/100 mL (HG) versus naturally contained in breast milk (about 4 IU/100 mL) and supplementation of 400 IU/day after 2nd week of life (LG)	HG: standard enteral formula LG: mothers milk with supplementation of vitamin D	10 weeks	BMD (g/cm^2)	BMD was higher in HG compared to LG at 10 weeks ($p < 0.001$)	NA

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
1999	Backstrom et al./ Finland	RCT	39	Not stated	Premature infants with GA < 33 weeks and appropriate weight for GA without major congenital malformation.	Vitamin D: 960 IU/day (HG) versus 200 to 400 IU/day (LG)	Enriched formula -Full enteral nutrition	6 months	BMC (mg) BMD (g/cm^2) Weight Length Head circumference	No significant differences between groups in BMC, weight, and length. ($p > 0.05$, $p = 0.23$, and $p = 0.41$, respectively)	Weight (median, HG: 7600 g versus LG: 7545 g) and length (median, HG: 67.4 cm versus LG: 66.3 cm) at 6 month of corrected age ($p = 0.23$ and $p = 0.43$, respectively). Head circumference seemed to be significantly higher in HG compared to LG (median, HG: 44.5 cm versus LG: 43.4 cm) ($p = 0.05$).
1999	Cooke, et al./UK	RCT (12 months)	103	60 males 43 females	Preterm infants (gestational age ≤34 weeks) and birthweight (≤1750 g), without evidence of systemic disease, requiring no medication, and growing normally at the time of hospital discharge; i.e.,≥25 g/d.	Ca: 108 mg/100 mL (135 mg/100 kcal) (HG) versus 54 mg/100 mL (82 mg/100 kcal) (LG) P: 54 mg/100 mL (68 mg/100 kcal) (HG) versus 27 mg/100 mL (40 mg/100 kcal) (LG)	HG1: enteral preterm formula until 6 months GA LG: enteral term formula until 6 months GA	12 months	BMD (g) BMM (g) BMM gain (g/d) Bone area (BA) (cm^2) BA gain (cm^2/d) Weight	No differences were detected in BMD in both groups. BMM was higher in HG (compared to LG) in 6 and 12 months only among boys.	At 6 and 12 months corrected age, weight was higher in HG than LG only for boys (weight, HG:9645 ± 1255 g versus LG: 8683 ± 827 g) ($p < 0.0001$).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
2000	Faerk et al./Denmark	RCT (36 weeks of GA)	127	Not stated	Premature infants with GA <32 weeks and without major congenital malformations	Ca: maternal milk content (unspecified) (LG) versus maternal milk (HG1) versus maternal milk and 70 mg/100 mL (HG2) P: maternal milk content (unspecified) (LG) versus maternal milk and 10 mg/100 mL (HG1) versus maternal milk and 35 mg/100 mL (HG2) Vitamin D: 800 IU all groups	LG: mother's milk HG1: mother's milk and enteral supplement with 10 mg P/100 mL HG2: mother's milk and fortifier with 35 mg Ca/100mL, 17 mg P/100 mL and 0.4 g protein/100 mL		BMC (g) BA (cm^2) Weight Length Head circumference	No significant differences on BMC (LG: 42.2 (10.4), HG1: 47.5 (7.5), HG2: 46.9 (9.7)) ($p = 0.15$, and after correction for non-dietary factors $p = 0.18$).	No significant differences in weight ($p = 0.25$), length ($p = 0.28$), head circumference ($p = 0.82$).
						Ca: maternal milk content (unspecified) (LG1) versus 70 mg/100 mL (LG2) versus unspecified (LG3) versus milk or formula (HG1) versus milk or formula and 35 mg/100 mL (HG2) P: maternal milk content (unspecified) (LG1) versus 35 mg/100 mL (LG2) versus unspecified (LG3) versus milk or formula and 10 mg/100 mL (HG1) versus milk or formula and 17 mg/100 mL (HG2) Vitamin D: 800 IU all groups	LG1: own mother's milk LG2: preterm formula with 70 mg/100 mL Ca, 35 mg/100 mL P, 2 g/100 mL protein (100 mg/100 kcal Ca, 50 mg/100 kcal P) LG3: preterm formula and own mother milk (various analogies) HG1: mother's milk and/or high-protein bank milk and enteral supplement with 10 mg P/100 mL HG2: mother's milk and/or ordinary bank milk and fortifier with 35 mg Ca/100 mL, 17 mg P/100 mL and 0.4 g protein/100 mL	36 weeks		No significant differences on BMC (LG1: 42.2 (10.4), LG2: 49.9 (12.2), LG3: 49.5 (6.4), HG1: 45.7(8.7), HG2: 46.6 (9.9)) ($p = 0.10$, and after correction for non-dietary factors $p = 0.35$). There was a tendency of difference in LG1 and LG2 ($p = 0.05$), but after correction for non-dietary factors it was not significant ($p = 0.68$)	Significant differences in weight ($p = 0.02$), LG1: 3029 (537), LG2: 3419 (500), LG3: 3566 (650), HG1: 3157 (478), HG2: 3218 (503)). Only, LG1 and LG3 differed between groups ($p = 0.05$). No significant differences in length (0.11), head circumference (0.57).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
2002	De Curtis et al./Italy	RCT (2 months)	33	16 males 17 females	Premature infants with GA < 35 weeks and birth weight < 1750 g, with no clinical problems	Ca: 80 mg/100 mL (108 mg/100 kcal) (HG) versus 54 mg/100 mL (82 mg/100 kcal) (LG) P: 40 mg/100 mL (54 mg/100 kcal) (HG) versus 27 mg/100 mL (41 mg/100 kcal) (LG)	HG: Post-discharge formula LG: Standard term formula	2 months	BMC (g) BA (cm^2) BMD (g/cm^3) Weight Length Head circumference	No significant differences between groups in BMC ($p > 0.05$)	No significant differences between groups in weight (HG: 4200 ± 270 g versus LG: 4030 ± 539 g), length (HG: 52.8 ± 1.4 cm versus LG: 52.4 ± 2.7cm), and head circumference (HG: 37.7 ± 1.1 cm versus LG: 37.8 ± 1.8 cm) 2 months from the study interval ($p > 0.05$).
2004	Lapillone, et al./ France	RCT (10 weeks)	37	Not stated	Premature infants with gestational age 28–32 weeks, birthweight 1000—1600 g, whose size was appropriate for gestational age, who were medically stable, and whose mothers had decided against breast-feeding were eligible for inclusion, provided that full formula feeding was established by 3 week of age.	Ca: 100 mg/100 mL (124 mg/100 kcal) (HG) versus 80 mg/100 mL (99 mg/100 kcal) (LG) P: 60 mg/100 mL (74 mg/100 kcal) (HG) versus 42.5 mg/100mL (52 mg/100 kcal) (LG) Vitamin D: 9.7 IU/100 mL (HG) versus 6 IU/100 mL (LG)	HG and LG: enteral formulas with different nutrient composition	10 weeks	BMC (g) BMC (g/kg per body weight) Weight Length Head circumference	BMC (measured in g and g/kg) was significantly higher in HG than in LG ($p < 0.05$)	Weight gain was significantly higher in HG (mean: 2727 ± 565 g, 95%CI: 2455, 2999 g) than in LG (mean: 2337 ± 332 g, 95%CI: 2172, 2502 g) at expected term (3 months of age) ($p = 0.016$). Length [HG: 1.3 ± 2.2 cm (95%CI: 10.3, 12.4) versus LG: 10.7 ± 1.7 cm (95%CI: 19.8, 11.5)] and head circumference gain [HG: 9.4 ± 1.4 (95%CI:8.7, 10.0) versus LG: 8.5 ± 1.7 cm (95%CI: 7.6, 9.3 cm)] remained similar between feeding groups ($p > 0.05$).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
2006	Koo et al./ USA	RCT (follow-up 12 months)	89	Not stated	Formula-fed preterm infants with GA 34 week or less, with intact gastrointestinal tracts and tolerated with full enteral feeding. Exclusion criteria included the presence of major congenital malformation, history of gastrointestinal surgery, or severe postnatal complications and adequate enteral feeding at the time of enrolment	Ca:105 mg/100 kcal (HG) versus 73 mg/100 kcal (LG) P: 62 mg/100 kcal (HG) versus 56 mg/100 kcal (LG) Vitamin D: 60 IU/100 kcal (LG) versus 80 IU/100 kcal (HG)	HG: nutrient enriched formula (EF) LG: Term formula (TF)	12 months	BMC (g) Weight Length Head circumference	LG significantly higher BMC compared to HG ($p < 0.051$).	Weight gain significantly higher in LG (*z*-score = 0.011 ± 0.0017) compared to HG (0.006 ± 0.0007) 1 year after hospital discharge ($p = 0.006$) Length gain was higher in LG (*z*-score = 0.016 ± 0.0012) compared to HG (*z*-score = 0.012 ± 0.0007) ($p = 0.04$), while head circumference remained similar among the feeding groups (*z*-score: HG:0.012 ± 0.0013 versus LG: 0.014 ± 0.0016) ($p > 0.05$).
2007	Litmanovitz, et al./ Israel	RCT (6 months)	20	10 males 10 females	Infants with birthweight <1500 g and appropriate for gestation age if they were fed only with formula. Preterm infants with severe central nervous system disorder, major congenital anomalies, chronic lung disease, or a prior diagnosis of necrotizing enterocolitis were excluded.	Ca: 78.1 mg/100 mL (105/100 kcal) (HG) versus 52.7 mg/100 mL (78 mg/100 kcal) (LG) P: 46.1 mg/100 mL (62 mg/100 kcal) (HG) versus 28.4 mg/100 mL (42 mg/100 kcal) (LG) Vitamin D: 52.1 IU/100 mL (HG) versus 40.5 IU/100 mL (LG)	HG: enteral post-discharge formula LG: enteral term formula	6 months	Bone SOS (m/s) Weight Length Head circumference	Bone SOS measurements, did not differ between groups at 3 and 6 months ($p > 0.05$).	Weight (HG:6948 ± 975 g versus LG:7313 ± 694 g) length (HG:65.9 ± 3.6 cm versus LG: 65.6 ± 2 cm) and head circumference (HG: 43 ± 1.8 cm versus LG:44 ± 2.2 cm) remained similar among feeding groups at 6 months of corrected age ($p > 0.05$).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
2008	Picaud et al./France	RCT (follow-up 12 months)	42	Not stated	Preterm infants with GA of 33 weeks or less, birth weight < 1750 g, and no major congenital malformations. Only infants fed exclusively with formula at the time of discharge were eligible for study inclusion.	Ca:100 mg/dL (123 mg/100 kcal) (HG) versus 51 mg/100 dL (76 mg/100 kcal) (LG) P: 53 mg/100 dL (65 mg/100 kcal) (HG) versus 28 mg/100 dL (42 mg/100 kcal) (LG) Vitamin D: 8 IU/100 mL (HG) versus 4 IU/100 mL (LG)	HG: Preterm formula (PF) LG: Term formula (TF)	12 months	BMC (g) BMC (g/kg) BA (cm^2) BMD (g/cm^2) Weight Length Head circumference	BMC significantly higher in HG compared to LG at 4 months after discharge ($p = 0.01$).	Significant higher values in HG compared to LG in weight (HG: 9486 ± 1310 g versus LG: 8479 ± 1189 g, $p = 0.01$), length (HG:74.7 ± 4.2 cm vs LG: 72.0 ± 3.6 cm, $p = 0.01$), and head circumference (HG: 46.5 ± 1.9 cm vs 45.3 ± 1.8 cm, $p = 0.02$), 12 months post-term.
2011	Pereira-Da-Silva, et al./Portugal	RCT (6 weeks of birth)	86 (17 and 17 at 6 weeks)	38 males 48 females	Preterm infants with GA of 33 weeks or less and requiring PN for at least 1 week, without major congenital abnormalities, severe central nervous system disorders, and bone and/or muscular diseases	Ca: 75 mg/kg/day (73 mg/100 mL or kcal) (HG) versus 45 mg/kg/day (44 mg/100 mL or kcal) (LG) P: 44.1 mg/kg/day (43mg/100mL or kcal) (HG) versus 26.5 mg/kg-1/day-1 (25.8mg/100mL or kcal) (LG) Vitamin D: 160 IU/kg/day (HG and LG) (both: 90-115 kcal kg/day)"	HG: PN solution LG: PN solution (in both when EN could be initiated the same feeding regimen was used in both groups)	6 weeks	Bone SOS (m/s)	SOS measurement declined at LG group, but remained steady at HG group at 6 weeks. At 6 weeks SOS measurement was lower in LG: 2853 (139) than HG: 2959 (123) ($p = 0.011$)	NA
2012	Van de Lagemaat et. al/Netherlands	RCT (6 months)	93	50 males 43 females	Preterm infants with GA of 32 weeks or less and birthweight 1500 g or less, without congenital malformations or conditions known to affect growth or body composition.	Ca: 65 mg/100 mL (97 mg/100 kcal) (HG) versus 50 mg/100 mL (75 mg/100 kcal) (LG) P: 38 mg/100 mL (57 mg/100 kcal) (HG) versus 30 mg/100 mL (45 mg/100 kcal) (LG) Vitamin D: 56 IU/100 mL (HG) versus 48 IU/100 mL (LG)	HG: post-discharge formula LG: term-formula (term age to 6 months)	6 months	BMC (g) BA (cm^2) Weight Length	BMC significantly higher in HG compared to LG at 6 months after term age ($p < 0.05$).	At 6 months of corrected age, similar values of weight (HG: 7380 g versus LG: 7460 g) and length (HG: 67.0 cm versus LG:66.5 cm) ($p > 0.05$).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
2014	Natarajan et. al/India	RCT (follow-up 3 months of corrected age)	87	Not stated	Preterm infants born between 28 and 34 weeks' GA and receiving at least 100 mL/kg per day of enteral feedings by 2 weeks' postnatal age. Infants with major malformations, those who received PN for 2 weeks or more, or those whose mothers receiving phenytoin therapy or presented HIV infection were excluded.	Vitamin D: 800 IU/day (HG) versus 400 IU/day (LG)	Drug administered directly to participants or mixed in breast milk or preterm formula	3 months	BMC (g) BMD (g/cm^2) Weight Length Head circumference	No significant differences between groups in BMC, ($p = 0.27$).	No significant differences in weight (HG: 4770 ± 820 g versus LG: 4825 ± 1053 g, $p = 0.79$), length (HG: 57.0 ± 3.4 cm versus LG: 57.8 ± 4.0 cm, $p = 0.35$) and head circumference (HG:38.0 ± 1.82 cm versus LG: 38.6 ± 1.7 cm, $p = 0.12$) at 3 months of corrected age.
2017	Anderson-Berry et al./USA	RCT (8 weeks)	32	Not stated	Patients with GA <32 weeks. Exclusion criteria included infants with congenital abnormalities, gastro-intestinal, liver, or kidney disease, parathyroid disease, calcium metabolism disorders, and infants receiving seizure medication or steroids.	Vitamin D: 800 IU/day (HG) versus 400 IU/day (LG)	Enteral vitamin D from breast milk and human milk fortifier or preterm formula.	8 weeks	BMC BMD Weight Length Head circumference	A higher proportion of infants in the 400 IU group had bone mineral content (BMC) measurements <10 percentile (400 IU 56% vs 800 IU 16%) ($p = 0.04$).	No significant differences between groups in weight (HG: 2542 g versus LG: 2378.5 g) and head circumference (HG: 33.25 cm versus LG: 32.1 cm) ($p = 0.23$ and $p = 0.75$, respectively) were identified at 35 weeks of life. In HG, length tended to be significantly higher compared to LG (HG: 45.75 cm versus LG: 43 cm) ($p = 0.09$).

Table 1: (continued)

Year	Author/country	Study design and duration	Number of participants (*n*)	Gender	Participants characteristics	Mineral and dosage	Route of administration	Duration of intervention	Outcome measures	Key findings-bone health (and time-points of follow-up measures if they differ from intervention end-point)	Key findings-growth indicators
2017	Mazouri et al./Iran	RCT (4 weeks)	50	32 males 18 females	Preterm infants with gestational age <32 weeks and weight <1500 g, without history of maternal hyperparathyroidism or maternal vitamin D deficiency. Parental consent was necessary.	Added Ca (Glycophos): 1.5 mmol/kg/day (HG) versus 0mg/100 mL (LG)	HG: Total PN with intravenous sodium glycerophosphate (Glycophos) LG: Total PN without intravenous sodium glycerophosphate.	4 weeks	BMD (g/cm^2)	Higher BMD in HG, compared to LG ($p < 0.001$)	NA
1995	Schanler et al./USA	Retrospective-cohort study (1 year of sample inclusion and about 6 to 8 weeks follow up)	26	Not stated	Premature infants with birthweight less than 1.500 g, appropriate size for GA, their mother intended to breastfeed, and without cardiopulmonary and gastrointestinal disorders	Ca: 90 mg/4 g powder (136 mg/100 kcal) (HG) versus 60 mg/4gm powder (97 mg/100 kcal)(LG) P: 45 mg/4 g powder (94 mg/100 kcal) (HG) versus 33 mg/4 g powder (52 mg/100 kcal) (LG) Vitamin D intake: 550 IU/day (HG) versus 1000 IU/day in (LG)	HG: Calcium gluconate-glycerophosphate LG: Calcium phosphate	1 year	BMC (mg/cm) Weight	The difference between groups was significant regarding BMC ($p = 0.002$), at 6–8 post intervention.	Similar values of weight (HG: 1498 ± 250 g versus LG: 1507 ± 166 g) among feeding groups at the end of treatment administration (balance study) ($p > 0.05$).

Where possible, all measurement units of feeding regimens were converted into mg/100 kcal, based on the average daily energy intake or average mg/kg or daily volume intake (milk/formula/fortifier). The conversions are reported in a parenthesis

Abbreviations: *GA*-gestational age, *HG*-High-dose Group, *LG* Low-dose group, *RCT* randomized control trial, *NA* not appliable (not measured).

Table 2 Quality assessment of included studies using PEDRO scale for RCTS and JBI checklist cohort studies

PEDRO scale Main author, year	Eligibility criteria	Randomly allocated sample	Allocation concealment	Similar baseline characteristics	Subject blinding	Therapists blinding	Assessors blinding	Key outcomes available for >85% participant	All outcome available for all participant/ intention to treat analysis	Between group comparisons	Point and variability measures	Total score*
Bishop et al., 1993	X	X		X	X	X			X	X	X	7
Chan et al., 1993		X		X	X			X	X	X	X	7
Prestridge et al., 1993	X	X	X	X	X	X	X	X	X	X	X	10
Backstrom et al., 1999	X	X		X	X		X	X	X	X	X	8
Cooke et al., 1999 (citation)	X	X		X	X			X	X	X	X	7
Faerk et al., 2000	X	X	X	X	X	X	X		X	X	X	9
De Curtis et al., 2002		X		X	X			X	X	X	X	7
Lapillone et al., 2004	X	X	X	X	X	X		X	X	X	X	9
Koo et al., 2006	X	X	X	X	X	X	X		X	X	X	9
Litmanovitz et al., 2007	X	X	X	X			X	X	X	X	X	8
Picaud et al., 2008	X	X	X	X	X	X	X	X		X	X	9
Pereira-Da-Silva et al., 2011	X	X		X	X	X	X	X	X	X	X	9
Van de Lagemaat et al., 2012	X	X	X	X	X	X	X	X		X	X	9
Natarajan et al., 2014	X	X	X	X	X	X		X	X	X	X	9
Mazouri et al., 2017	X	X		X			X	X	X	X	X	7
Anderson-Berry et al., 2017	X	X	X	X	X	X	X	X	X	X	X	10

Table 2 (continued)

PEDRO scale Main author, year	Eligibility criteria	Randomly allocated sample	Allocation concealment	Similar baseline characteristics	Subject blinding	Therapists blinding	Assessors blinding	Key outcomes available for >85% participant	All outcome available for all participant/ intention to treat analysis	Between group comparisons	Point and variability measures	Total score*
JBI Critical Appraisal Checklist for Cohort Studies Main author, year	Similar population groups	Same exposure measurements	Exposure measured in valid and reliable way	Confounding factors identified	Strategies to deal with confounders	Participants free of the outcome at baseline	Outcomes measured in a valid and reliable way	Follow up was sufficient	Follow up complete/if not reasons addressed	Strategies to address incomplete follow up	Appropriate statistical analysis	Total score
Schanler et al., 1995		X	X			X	X	X	X	X	X	8
Alpay et al., 1998	X	X	X		X	X		X	X		X	8

X translates to yes/the question was answered positively and sufficiently. Only RCTs with score higher than 7 and cohort studies with score at least 8 are shown

*Eligibility criteria do not count towards the final score for RCTs

Type of administration

Only 3 studies followed PN regimens. Eleven studies administered the minerals and vitamins via enteral formulas, 2 via mother's milk fortifier and 2 via preterm formula or breast milk fortifier.

Follow-up duration

The key outcome of bone health indicator was assessed at 1 month of life ($n = 4$ studies), 2 months ($n = 3$), 3-4 months following the start of the intervention ($n = 3$), following 6 months ($n = 4$), following 9 months ($n = 1$), or at 12 months ($n = 3$).

Main outcomes on bone mineral content

Studies combining elevated Ca, P, and vitamin D and BMC

All 7 studies on EN in which Ca, P, and vitamin D intake was higher in HG, presented significant differences in BMC. Average BMC was higher in HG after various durations of follow-up: between discharge and expected term (HG: 53.6 ± 21.7 g, LG: 38.9 ± 17.7 g, $p = 0.037$) [28] at hospital discharge (age adjusted: HG: 42 ± 4 mg/cm, LG: 31 ± 5 mg/cm, $p = 0.002$) [29] 4 months after inclusion (HG: 104.4 ± 29.2 g, LG: 87.5 ± 17.1 g) ($p = 0.01$) [30], 6 months after term age ($p < 0.05$) [9], and at 3 and 9 months after discharge (9 months mean difference: 26.8 mg/cm, 95%CI: 13.2, 40.4) [31]. In one study, BMC was reported to differ between HG and LG at 8 weeks after discharge ($p < 0.001$), but not at 16 weeks [32]. Only one study found significantly higher BMC in LG at 12 months of age [27].

Studies combining elevated Ca and P (non-varying vitamin D) and BMC

Among the 4 studies, 2 EN and 2 PN regimens, in which only Ca and P were higher in HG, and not vitamin D, the effect on BMC was less consistent. Only in two studies, a positive effect was evident in both boys and girls on PN (HG: 2.7 ± 0.6, LG: 2.0 ± 0.50 mg/cm/week, $p = 0.025$) [33] or just in boys on EN regimens (significant BMC gain: BMM, HG: 202 ± 35g, LG: 186 ± 23g, $p < 0.01$) [34]. In contrast, in one EN study, BMC remained similar among the two groups, 2 months after the study initiation ($p > 0.05$) [35]. A tendency of difference in BMC was visible among infants receiving own mothers milk and those receiving PF ($p = 0.05$), but after some adjustments the difference was not significant ($p = 0.68$) [25].

Vitamin D and BMC

Out of the three EN studies, in which only vitamin D intake differed (not Ca or P), only one study resulted in a beneficial effect in BMC. This study [36] presented a higher proportion of infants in the HG (HG: 800 IU/day, LG: 400 IU/day) having BMC measurements <10 percentile at 8 weeks ($p = 0.04$). The remaining two studies found no difference ($p > 0.05$) at 3 [37] and 6 months [38].

Main outcomes on BMD and SOS

BMD was increased in infants receiving higher doses of Ca, and/or P and/or vitamin D after 4 weeks of follow up (HG: 0.13 ± 0.01 mg/cm^2, LG: 0.10 ± 0.02 mg/cm^2, $p < 0.001$) (22), 6 months after term age (HG: 0.221 ± 0.028 g/cm^2, LG: 0.198 ± 0.019 g/cm^2) and after 10 weeks of life (HG: 0.198 ± 0.018, LG: 0.144 ± 0.013 g/cm^2, $p < 0.001$) [39].

Regarding SOS measurements, two studies found contradicting results regarding EN and PN, as SOS measurements were higher in HG at 6 weeks in one study following a PN regimen (HG: $2\,959 \pm 123$ m/s, LG: $2\,853 \pm 139$m/s, $p = 0.011$) [40], but not at 6 weeks in another study following a EN regimen (HG: $3\,032 \pm 60$ m/s, LG: $2\,978 \pm 83$ m/s, $p > 0.05$) [41].

Analysis of administered dosed in light of bone mineralization improvement

Ca administration across EN studies effective at improving bone health indicators ranged between 97 and 136 mg/100 kcal. When doses of 192 against 82 mg/100 kcal were examined [32], BMC did not differ at 16 weeks. P administration in HGs across effective EN interventions displayed high variance: 49–94 mg/100 kcal. Among them, some compared doses in the higher end of this range (in HGs) with those at the lower end (in LGs), with range 57–94 mg/ 100 kcal, which were effective at improving bone health indicators [9, 28–30]. Studies on PN or vitamin D administration were fewer, limiting the discussion of the administered doses.

Meta-analyses

The meta-analysis model assessing the beneficial effect of (A) higher Ca and P doses (not vitamin D); (B) higher Ca, P, and vitamin D doses; and (C) higher vitamin D doses among groups can be found in Fig. 3. Four studies did not present or the exact average BMC or BMD, with the corresponding variance could not be calculated and thus were not included in the models. Higher doses of Ca and P resulted in increased bone mineralization for both EN and PN studies, with the effect being more evident for PN studies (standardized mean difference: 0.88; 0.34, 1.43 with $I^2 = 0\%$), than EN (0.39; 0.09, 0.69 with $I^2 = 0\%$). After combining the EN and PN results, a clear benefit of higher Ca and P doses was evident for bone mineralization (0.51; 95% CI 0.24, 0.77 with $I^2 = 0\%$). A similar effect on EN was higher in premature infants receiving higher doses of all three elements (1.72; 0.81, 2.16), although high heterogeneity was noted ($I^2 = 90\%$, $p < 0.0001$). Higher intake of vitamin D through EN (but not Ca or P) did not result in increased bone mineralization (−0.01; −0.59, 0.56 with $I^2 = 53\%$). Higher doses of Ca and P, regardless of vitamin D doses, predicted an increased bone mineralization (EN: 1.18; 0.67, 1.69 with $I^2 = 85\%$) (Supplementary Figure 1).

Growth indicators

Growth indicators were measured only in EN studies. Regarding weight, only in 4/13 studies, did higher doses of Ca, P, and/or vitamin D present a positive effect on weight gain [25, 28, 30, 34], while another study provided evidence of an opposite effect [27] (Table 1). In 8/13 studies, weight values remained similar among feeding groups [9, 29, 32, 35–38, 41]. Length gain did not differ in 8/11 studies [9, 25, 28, 32, 35, 37, 38, 41], while two indicated significantly higher gain in HGs [30, 36] and one in LGs [27]. Head circumference presented similar values across 7/9 studies [25, 27, 28, 35–37, 41]., with only two suggesting a favorable effect in HGs [30, 38].

Discussion

Summary of key results

In a sample of mostly VLBW premature infants, we found that the overall effect of higher doses of Ca and P on bone mineralization in premature infants is likely positive, while higher dose of vitamin D on its own is unlikely to be beneficial in increasing BMC. The effect of higher doses of all three components was almost unanimous in augmenting BMC. The current meta-analysis has confirmed the aforementioned, and suggest that higher doses of Ca and P administered either through EN or PN can ameliorate the diminished bone mineralization in premature infants. The beneficial effect of higher EN doses of Ca, P, and vitamin D on weight, length, and head circumference of premature infants is improbable. Our findings suggest that premature infants receiving EN or PN may benefit from increased doses of Ca and P. According to the studies included in our review, to ensure an adequate bone mineralization, a beneficial enteral dose may be in the range of Ca: 95–135 mg/100 kcal and P: 55–95 mg/100 kcal, accompanied by sufficient doses of vitamin D

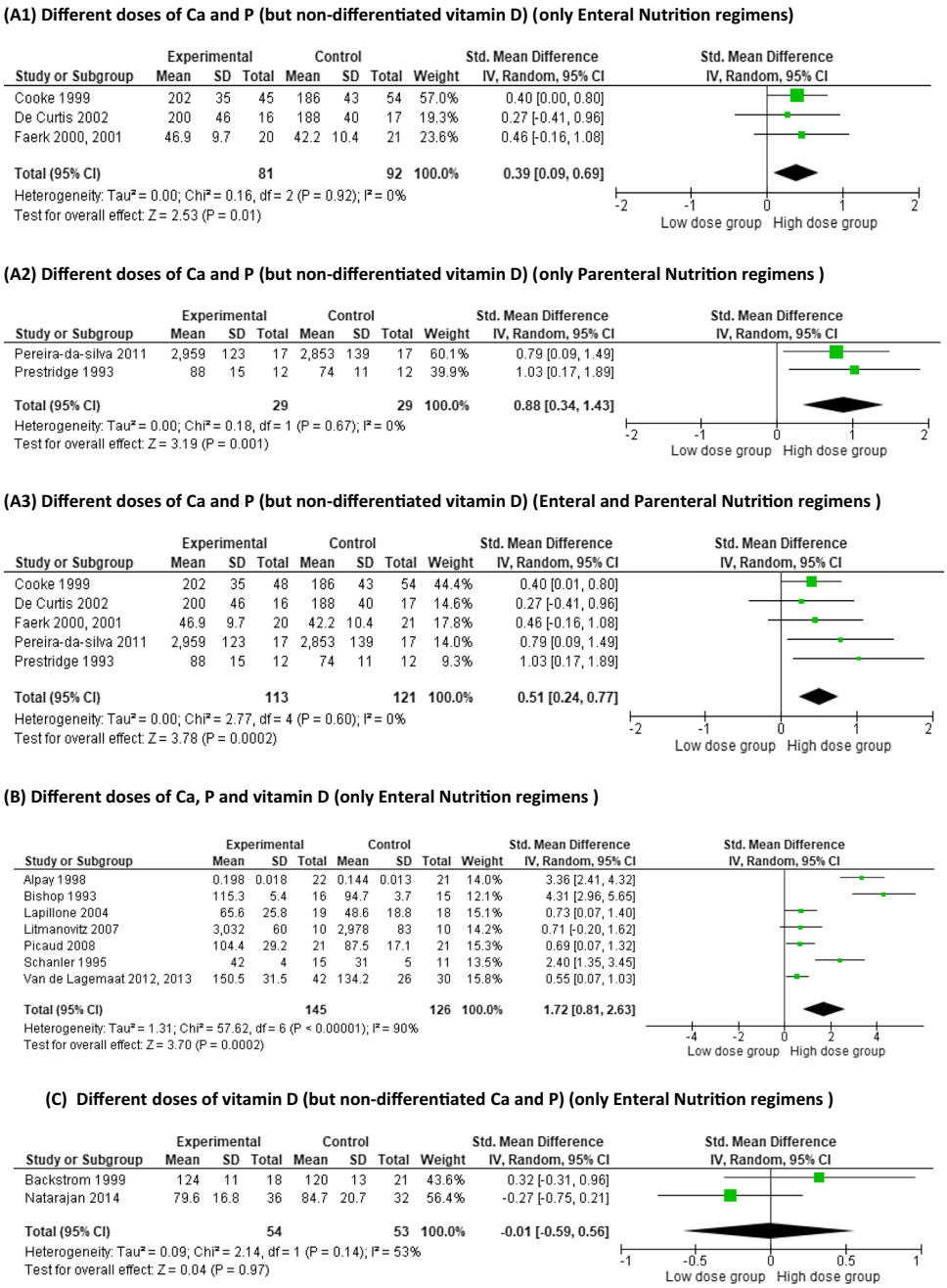


Fig. 3 Forest plots of comparison: the outcome of higher compared to lower doses of Ca, P, and/or vitamin D in bone mineralization. Footnote for all figures: All included studies evaluated BMC, apart from Cooke 1999 and Alpay 1998 (BMD), and Pereira-da-Silva 2011 and Litmanovitz 2007 (Bone SOS). As outcome measures differed, but all was indicative of bone mineralization, the standardized mean difference random effect models were used. BMC, bone mineral content; BMD, bone mineral density; SOS, speed-of-sound; 95% CI, 95% confidence interval

Ca and P in bone health

AAP recommends 140–160 mg/100 kcal Ca and 95–108 mg/100 kcal P [11], administered through fortified human milk or preterm formula, while ESPGHAN recommends EN doses of 70–140 mg/100 kcal Ca and 50–82 mg/100 kcal P [10, 42]. Hence, ESPGHAN recommendations are more in line our findings. However, we could not assess the effectiveness of AAP recommendations with certainty as only one study included ranges of doses supported by AAP. For PN solutions, as supported by our meta-analysis, the ideal doses of these minerals might be lower, since minerals are directly available for bone mineralization and tissue accretion and thus present better absorption [43].

Vitamin D in bone health

Apart from infants with extremely low BMC at baseline, vitamin D alone could not improve bone mineralization, even when accounting for various ranges of administration. Clinical organization presents disparities regarding the recommended doses, as the European guidance support higher doses (up to 1000 IU/day) [10] to ensure high Ca absorption [44], while AAP support lower doses (400 IU/day) [11] to

prevent conditions such as hypercalcemia, hypercalciuria, and nephrocalcinosis [45].

Growth indicators

Elevated doses of Ca, P, and/or vitamin D might not influence growth indicators improvement. Significant findings across studies may be attributed to disparities in energy and protein intake or the limited capability of preterm infants to regulate their energy intake [28, 34]. Nevertheless, a systematic review has noted that 800–1000 IU/day of vitamin D may benefit length gain and head circumference [46]. As this study did not systematically search growth indicators, we believe that this review's findings [46] present higher validity.

Limitations and strengths

High heterogeneity regarding the doses of minerals and vitamins, administration type, intervention and follow-up duration, age, birthweight, and outcomes reported was present. The administered nutrients forms were stated only in limited studies, with gluconate Ca, glycerophospate, and hydroxyl-vitamin D being the most common. Therefore, we could not identify the precise doses and forms of the examined nutrients. The total amount of administered nutrients, and in particular of vitamin D, was lower than the recommended ranges in most studies, limiting the credibility of our findings, regarding the effect of higher doses on bone mineralization. Studies following a PN regimen, although highly effective, were limited (3), and therefore, no mineral range was provided. Additionally, the meta-analysis combining the results of both EN and PN regarding Ca and P administered doses has significant limitations related to the core differences of EN and PN, in terms of administration routes and absorption rate. However, by simultaneously accounting for the effect of elevated does of Ca and P in both EN and PN (Figure 3)A3, we were also able to provide clear indications about the well-known beneficial effect of these nutrients on bone mineralization, regardless of the feeding regimen. Biochemical markers of metabolic bone disease, which can provide crucial early signs of MBD, such as serum biochemical biomarkers (ALP, Ca, P), were not assessed as primary outcomes in our study since they are not specific MBD predictors or exact measures of BMC in prematurity [8]. Analyzing the effect on bone mineralization based on these indicators as outcome measures may prove challenging due to the difficulty in incorporating these results into meta-analysis models and as they are often not considered the best option for diagnostic purposes but primarily for predicting poor bone health in the hospital setting or primary prevention. We chose to only include measurements with less variation attributed to human or device errors and focused mainly on measurements obtained from gold standard methods. Finally, growth indicators as outcomes were not systematically reviewed.

Conclusion

The identified studies suggested a possible beneficial effect of supplementary doses of Ca (EN: 95–135 mg/100 kcal) and P (EN: 55–95 mg/100 kcal) on bone mineralization among preterm infants. Vitamin D can enhance the beneficial effect of these minerals. Disparities in the recommended doses of minerals and vitamins can introduce challenges across neonatal units. This review identifies potential areas for further research, including the use of specific formulas for administration of Ca, P, and vitamin D; the monitoring of formula intake from parents or caregivers; and the establishment of the optimal duration of supplementation.

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Author contributions A Vervesou: conceptualization, methodology, data collection, data synthesis, meta-analysis, investigation, resources data curation, writing original draft, project administration. DV Diamantis: methodology, data collection, data synthesis, meta-analysis, investigation, data curation, writing review, and editing. K Maslin, JH Carroll: conceptualization, methodology, writing review and editing, supervision.

Declarations

Ethics approval An ethics statement is not applicable because this study is based exclusively on published literature.

Consent to participate Not appliable

Consent for publication Not appliable

Competing interests The authors declare no competing interests.

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